extending from the Perdido River (reported but unexamined colony) in Escambia County to the Ochlockonee River drainage, Gadsden County. The distributional pattern suggests that dispersal of animals from Alabama and Georgia may have contributed significantly to the Florida population, although some of the existing colonies in the state may be descendants of the native stock. Three introductions are recorded.

Ecological notes on 6 active colonies are presented. All were located on small streams in, with one exception, wet or moist hardwood associations. Bank burrows were present at all sites and lodges at 3. Eighteen species of plants were recorded as having been utilized for food.

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DIETARY COPPER AND ENZYMES IN RABBIT SEMEN

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Numerous *in vitro* tests have been used to evaluate the quality of semen such as the determination of motility, concentration of sperm, dehydrogenase activity based on rate of reduction of methylene blue and other dyes, fructolysis, and phosphatase activity. White (1955) reported that copper was highly toxic to sperm motility when added *in vitro* and that glutathione when similarly added would alleviate the toxicity. MacLeod (1951) discussed the action of copper as being one of oxidation of thiol groups of dehydrogenases and suggested that a substance like glutathione would be a competitor for the copper or would reduce the thiol groups of the enzymes to their active state.

The present study was made to determine if high levels of dietary copper would exert an *in vivo* effect on the semen of rabbits in such a manner that it would influence motility and enzyme activity of semen comparable to copper added *in vitro*.

Experimental

Twenty New Zealand White male rabbits that averaged 16 months of age and 3,800 gm. of weight were divided into four dietary copper groups. Diets containing four levels of copper were prepared by spraying copper sulfate in solution on a commercial pelleted rabbit chow that contained 17 ppm of copper. The resulting diets contained 17, 37, 67, and 255 ppm copper by analysis. Assignment of males was made by random allotment of five to each of the four copper dietary groups. All rabbits were fed 100 gm of diet per day. Feed refused was weighed back weekly. All groups consumed about the same amounts of feed.

Semen was collected at two-week intervals. The five males in each dietary group were allowed to mate with a mature female and the semen was withdrawn with a catheter as a composite group sample. Semen was collected five times from each group. Semen volume and sperm motility and concentration were determined by conventional methods. Methylene blue reduction was measured according to Beck and Salisbury (1943) using 0.1 ml

of semen in a reaction volume of 1.2 ml. Glutamic-oxalacetic transaminase (GOT) activity was measured according to Sigma (1961).

After 73 days on the diets the rabbits were sacrificed. Testes, seminal vesicles, and liver were memoved and frozen at −8 C for subsequent analysis. Copper was determined by the Sandell (1959) procedure. Statistical methods used were from Snedecor (1956).

Results and Discussion

In table 1 the data are presented that were obtained on the semen for the effect of dietary copper on volume, motility and concentration of sperm, methylene blue reduction time expressed in minutes required for decoloration per 4,000 sperm in reaction mixture, and glutamic-oxalacetic transaminase activity expressed as Sigma-Frankel units per milliliter semen. A marked decrease ($P<.01$) in motility of sperm was observed at the 67 and 255 ppm levels of dietary copper. Volume of semen and sperm concentration were not affected. Methylene blue reduction time was increased ($P<.05$) at the 255 ppm dietary copper level. The treatments had no significant effect on the glutamic-oxalacetic transaminase activity. Those fed 37 and 67 ppm copper appeared to be stimulated and those fed 255 ppm copper appeared to have a decrease in activity. Gregoire et al. (1961) reported rabbit seminal plasma to have 329 (40-1120) units of GOT activity per milliliter. The higher values obtained in the present study may have been due to use of the whole semen for the analysis.

Values for the concentration of copper in the testes, seminal vesicles, semen, and liver are given in table 2. Dietary copper at levels of 37, 67, and 255 ppm increased ($P<.05$) copper in the testes, but only the 255 ppm level resulted in an increase ($P<.01$) in the seminal vesicles. There were no significant effects of the diets on the concentration of copper in the semen, even though the increased time for methylene blue reduction (see table 1) for the males that were on the high levels of dietary copper suggested that a significant amount of the inhibitory copper reached the semen from the diet. The copper level in the liver of the males fed 255 ppm copper was higher ($P<.01$) than that in the other three dietary groups.